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Age distributions of Greenlandic dwarf shrubs support concept of negligible actuarial senescence

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Abstract. Many plants and sessile animals may not show actuarial senescence, the increase in mortality with age predicted to be ubiquitous by classic evolutionary theories of aging. Age-structured demographic information is, however, limited for most organisms. We assessed the age distributions of nine dwarf shrub species from 863 taproot samples collected in coastal east Greenland. Penalized composite link models (pclm) were used to fill gaps in the observed age ranges, caused by low species-specific sample sizes in relation to life span. Resulting distributions indicate that mortality patterns are independent of age. Actuarial senescence is thus negligible in these dwarf shrub populations. We suggest that smoothing techniques such as pclm enable consideration of noisy age data for determining age distributions. These distributions may, in turn, reveal age effects on demographic rates. Moreover, age determination from the root collars of small plants constitutes a powerful technique to further investigate age dependency of the demography of many plant species, including eudicot herbs. Using these methods for long-lived plants where long-term monitoring is unrealistic, we show that age is unlikely to be an important variable for making population projections and determining extinction risks.

Key words: actuarial senescence; age distribution; age structure; Arctic dwarf shrubs; dendroecology; individual survival; mortality; penalized composite link model; plant aging; shrub demography.

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Introduction

It is now clear that age trajectories of mortality differ widely among taxa, and it appears that mortality of some species may be constant or even decrease with age (Jones et al. 2014). Actuarial senescence is the increasing risk of mortality with increasing age often observed in mammals and other animals. Determining age effects on mortality for a wide range of taxa is important for understanding the evolutionary background of actuarial senescence, and because of the potential implications of age-dependent demographic rates for population dynamics. Whether plants generally deteriorate physiologically as individuals grow old, with associated increases in mortality, is still unknown. There are a few studies indicating that such actuarial senescence may occur in herbaceous species, but most empirical studies have not found any support for this theory (reviewed in Dahlgren and Roach, *in press*).

For woody species such as trees and shrubs, it has been suggested that increases in plant size can lead to increases in mortality over age (Peñuelas 2005), possibly due to stress on the vascular system (Woodruff and Meinzer 2011, Thomas 2013). However, in most plant populations that have been monitored over longer periods, mortality was unrelated to the age of individuals (Sarukhan and Harper 1973, Dahlgren and Roach, *in press*). That this is also often the case for trees is supported by some analyses of age structures (e.g., Hett and Loucks 1976). Despite these studies, there is still a general lack of knowledge of demographic effects of plant aging because in plant demographic studies, size dependence rather than age dependence is typically assumed, and ages of plants often remain unknown (Caswell 2001, but see, e.g., Lauenroth and Adler 2008). Moreover, particularly little is known about the age-structured demography of shrubs (but see, e.g., Kuuseoks et al. 2001 for a study on the age distribution of aboveground stems in hazel, *Corylus* sp.).

To determine age trajectories of mortality of long-lived plants, such as shrubs, by monitoring individuals over their life courses is logistically infeasible. However, it is possible to determine ages of individuals based on growth increments in their root collars, that is, the oldest part of the plant where the root system transitions into the shoot system (Schweingruber and Poschlod 2005, Schweingruber and Büntgen 2013, Büntgen et al. 2014). Assuming that populations are close to a stable age distribution (with a constant long-term population growth rate) and that environmental drivers have not changed directionally over plant life courses, age distributions can be used to indicate types of survivorship curves (e.g., Caughley 1966). Nonlinear relationships between log individual density and age could result from intrinsic changes within individuals altering their mortality risks. However, such patterns could also be caused by several other processes, including environmental variation, density dependence, and differences in the risk of mortality between individuals. Linear relationships would indicate no effects of any of these factors, including that mortality is constant with age, unless different processes cancel each other out. So-called mortality plateaus in populations of humans and some other animals at high ages, for instance, have been suggested to reflect variations in average mortality among individuals masking within-individual increases in mortality with age, as more "frail" individuals gradually die (Vaupel and Yashin 1985, Steinsaltz and Evans 2004, Vaupel and Missov 2014).

Here, we determine ages for individuals of nine Greenlandic dwarf shrub species based on their annual growth increments. From previous analyses, it is known that climatic variation has triggered recruitment fluctuations over the life courses of the individuals in these populations (Büntgen et al. 2015). We now, for the first time, estimate smoothed age distribution functions for each species using penalized composite link models (Eilers 2007, Rizzi et al. 2015). We hypothesize that in the Arctic environment of these slow-growing dwarf shrubs, environmental effects on mortality of mature individuals would have been limited and not resulted in a directional change in mortality over time. Based on previous results with other plant growth forms, we also hypothesize that mortality is independent of age. In turn, we predict age distribution shapes to be log-linear for old individuals.

Methods

Data collection

Collection of individuals for age determination was carried out in coastal East Greenland, near Ittoqqortoormiit (70°26′6″ N and 21°58′100″ W). Temperature means and precipitation totals during the short growing season between mid-June and early August are 1.9°C and 94 mm (Schweingruber et al. 2013). The nine most abundant, circumpolar dwarf shrub species, *Arctostaphylos alpinus*, *Betula nana*, *Cassiope tetragona*, *Dryas octopetala*, *Empetrum nigrum*, *Rhododendron lapponicum*, *Salix arctica*, *Salix herbacea,* and *Vaccinium uliginosum*, were considered (see Schweingruber et al. 2013, Büntgen et al. 2015 for details). Dwarf shrubs were excavated independent of their size, which should have resulted in an unbiased sample of ages that reflected the age distribution of entire populations. All individuals of the study species present in each of 30 plots laid out over the study area were excavated. Sizes and locations of plots were decided with the aim of excavating at least 30 individuals per species. Excavation of intact above- and below-ground stem and root parts was targeted for each

individual. The oldest remaining part of the taproot was analyzed. The study species can reproduce clonally, and the ages of some individuals may thus reflect the date of ramet formation rather than seed germination. Cross sections were made with a sliding microtome, and annual growth increments in the xylem were determined using a light microscope. Annual growth increments were obvious as clear rings in the xylem, caused by the production of large vessels early in the growing season. Age estimates were obtained for a total of 863 of the sampled individuals (see Büntgen et al. [2015] for more details on the study area, sampling design, and age determination).

Statistical modeling

We used two different statistical smoothing techniques to describe age distributions and for hypothesis testing. First, we binned the data in age groups to remove noise caused by the small sample size relative to the long life spans of these species and fitted a penalized composite link model (Eilers 2007, Rizzi et al. 2015) to obtain good approximations of overall age distributions. Then, we used the individual-based data and fitted Poisson regression models to the data for the oldest individuals. We tested the statistical significance of linear and nonlinear parameters, modeled using restricted cubic splines (Harrell 2013).

The penalized composite link model (pclm) has been shown to be a powerful tool for ungrouping binned data in terms of human age-grouped data (Rizzi et al. 2015). The method is based on the composite link model, which in turn extends generalized linear models, with a penalty term added to ensure smoothness of the target distribution. The observed counts, in our case the number of individuals in a specific age group, are assumed to be Poisson-distributed and are treated as indirect observations of an ungrouped but latent sequence that is estimated via maximum likelihood. We use this model here as a smoothing method to retrieve shapes of the age distributions.

As there were no data for all ages, we pooled the observations into age bins. All species had unimodal age distributions, suggesting all available microsites are colonized and stable population sizes (entailing a stable population growth rate at $r = 0$; e.g., Knowles and Grant 1983, Johnson et al. 1994). We assumed that individuals older than the modal age had established in an environment with relatively low plant densities, and we included only the older individuals. We included only the older individuals also to focus on fully mature individuals, expected to have reached their full size, because before this age mortality likely decreased as the plants were growing. We started with 10-yr bins and increased bin sizes to 20, 30, 40, or 50 yr, until all age groups contained at least two observations, or until there were only three age groups (see Appendix S1). For the shorter-lived *Salix herbacea*, we used 5-yr bins. This resulted in smooth pclm estimations of age distributions for all species that were used to explore trends in age effects on mortality.

To corroborate the pclm results with formal statistical hypothesis tests of whether tail ends of age distributions suggested age dependence of mortality, we fitted log-linear curves using Poisson regression (corresponding to generalized linear models with a log link function and a Poisson-distributed error). We used the observed age data starting from the mode identified using the pclm analyses (i.e., the lowest ages in Fig. 1). Ages with zero observations were included when fitting the Poisson regressions, as regarding these as missing observations might have biased the fitted line due to a low number of individuals of very old age. The statistical significance of nonlinear relationships with age was estimated by fitting five-knot restricted cubic regression splines using the rms R package, and testing the effect of nonlinear terms using Wald tests (Harrell 2013). We also fitted second-degree polynomial models, but results were similar and are not presented for all species. All statistical analyses were carried out in R v.3.2.2 (R Core Team 2015).

Results and Discussion

The pclm analyses supported our hypothesis of negligible actuarial senescence in the nine studied Arctic dwarf shrub species. Age distributions for old individuals were approximately log-linear, with deviations from log-linearity varying among species and curves not being consistently either concave or convex (Fig. 1). This result was corroborated by the Poisson regressions, where nonlinear components were never statistically significant ($P > 0.2$ in all

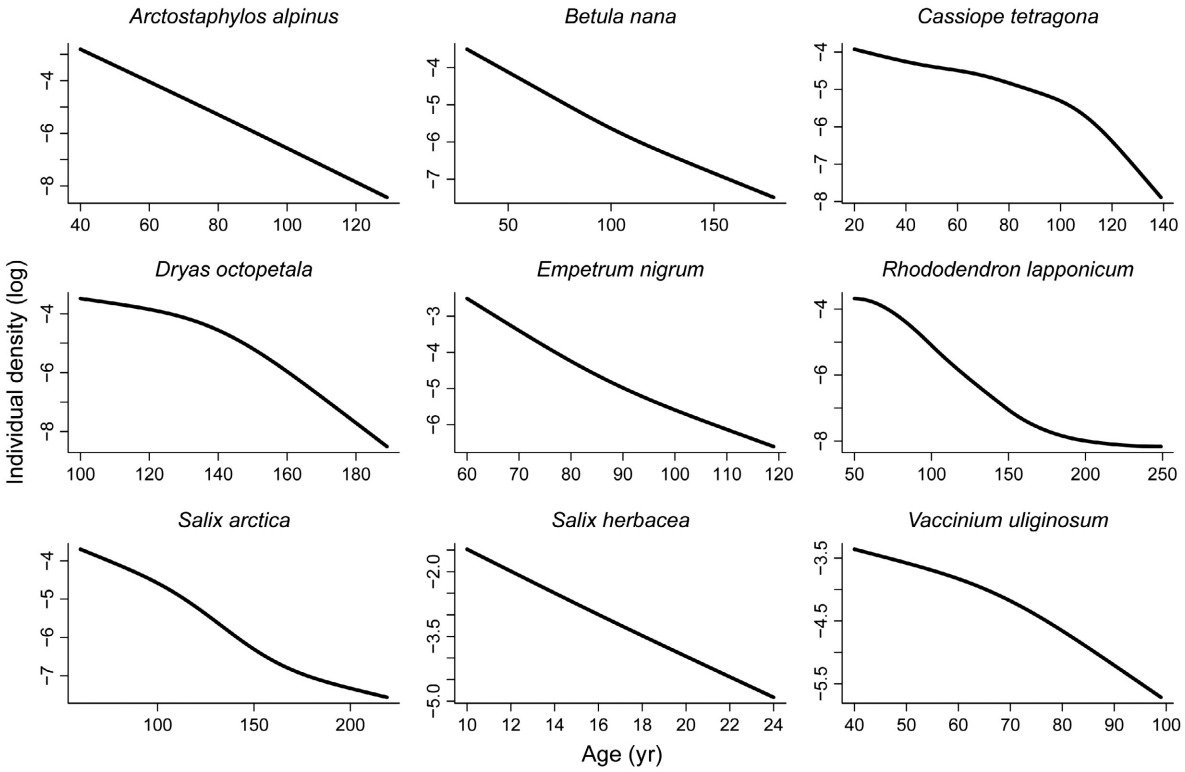

Fig. 1. The density of observed individuals (log) over age for nine Greenlandic dwarf shrub species. The minimum age per species is the observed mode of the age distribution. Curves were estimated using penalized composite link models (pclm). Note that axes vary among panels, but that all panels indicate the general shape of mortality over ages of adult plants as suggested by observed age distributions.

species; $P > 0.3$ in seven of nine species; Table 1). Linearized age effects on the other hand were clearly significant for all species. Slopes in the linear Poisson regressions ranged from −0.017 to −0.069 for the eight long-lived species, corresponding to a range of annual mortality rates of 1.7–6.7% for old individuals in a stable population. In the relatively short-lived *Salix herbacea*, the slope corresponded to an annual mortality of 18% after age 10 years. The identification of linear ("Type II"; Deevey 1947) log-survivorship curves indicates constant mortality. Log-linear patterns may also result from variation in individual frailty masking within-individual increases in mortality risk over age because frailer individuals will die at younger ages, or from positive or negative age effects being counteracted by environmental change. However, we would argue that the similar approximately log-linear relationships in all nine study species make both these processes less likely explanations of the observed patterns, and the most parsimonious interpretation of the results would be that no substantial effects of age on mortality occurred. This is in agreement with our predictions based on similar patterns in several other plant species (Sarukhan and Harper 1973, Dahlgren and Roach, *in press*), and the general consensus that plant demographic rates are more strongly size- than age-dependent (Caswell 2001, Horvitz and Tuljapurkar 2008).

Due to the low sample sizes, it cannot be ruled out that the nonlinear terms in the Poisson regressions reflected true age-dependent effects on mortality, despite not being statistically significant. It is therefore interesting to examine observed trends. In the Poisson regressions, *P*-values for nonlinear terms were relatively low for two species. For *Empetrum nigrum*, there was tendency ($P = 0.21$) suggesting decreased mortality with age (cf. Fig. 1). For *Dryas octopetala*, there was a tendency ($P = 0.27$) suggesting

Table 1. Summary statistics for Poisson regression models of individual density over age.

Species	Linear slope	Linear *P*-value*	Nonlinear *P*-value**
Arctostaphylos alpinus	−0.052	<0.0001	0.545
Betula nana	−0.025	<0.0001	0.729
Cassiope tetragona	−0.017	<0.0001	0.646
Dryas octopetala	−0.036	<0.0001	0.276
Empetrum nigrum	−0.069	0.0012	0.208
Rhododendron lapponicum	−0.035	<0.0001	0.548
Salix arctica	−0.027	<0.0001	0.522
Salix herbacea	−0.168	0.0006	0.322
Vaccinium uliginosum	−0.029	<0.0001	0.348

* *P*-values are based on Wald tests.
** Multiple Wald tests were carried out on all nonlinear parameters in five-knot restricted cubic spline regressions.

increased mortality with age. Still, if this relationship reflects actuarial senescence in *D. octopetala*, then it may indeed be regarded as "negligible." The slope of a second-degree polynomial term was only −0.00034, corresponding to an increase in annual mortality over 100 yr of 3.4%. The tendency of age dependence in mortality for these two species in the regression analysis was corroborated by the pclm analysis, and based on Fig. 1, weak increases in mortality with age may have occurred also in *Cassiope tetragona* and *Vaccinium uliginosum*. In addition, a few of the individual density–age relationships indicated very weak decreases in mortality with age. Overall, however, remaining distributions were either linear after a potential increase after the "hump" around the modal age or contained additional local maxima which may reflect past recruitment bursts (cf. Büntgen et al. 2015). The trends of changes with age illustrate that further research is needed to validate that actuarial senescence is negligible in these dwarf shrubs. In future studies, it would be particularly interesting to correlate mortality patterns with other aspects of life history or environmental conditions if there is variation among species. We do not attempt such comparisons here, however, due to the weak relationships.

We conclude that the available data support that actuarial senescence is negligible in nine stress-tolerant and long-lived Greenlandic dwarf shrubs. A lack of actuarial senescence in plants contradicts classical theories of aging based on expected selection pressures (Hamilton 1966). It does not contradict the disposable soma theory of aging, which predicts actuarial senescence in all organisms with clear early-life separation of the germ line from somatic cells (Kirkwood and Holliday 1979); as such, a separation is not clear in plants. A lacking indication of senescence does support the proposition that indeterminately growing organisms may avoid senescence (Vaupel et al. 2004). Moreover, this result indicates that age structures may not have to be accounted for when estimating population growth rates of shrubs, for example, when determining extinction risks of threatened species (cf. Morris and Doak 2002).

The age-determination method and the statistical pclm method used here should both constitute highly useful tools in future studies of aging in species for which no long-term data exist. The age-determination technique based on growth rings in root collars is not applicable only to plants that form woody aboveground stems but also to many eudicot herbs that form growth rings in the root xylem in seasonal environments (Schweingruber and Poschlod 2005). There is thus a large untapped pool of readily available data on age structures of herbs. There is also a large potential in analyzing existing age data on various organisms utilizing pclm to reconstruct age distributions if exact ages of individuals are unknown or uncertain, or if data are sparse. To obtain sufficient data to allow investigation of mortality patterns across large taxonomic scales, these and similar age-determination and statistical methods may be necessary.

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Supporting Information

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